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OBSERVATIONS ON THE TRANSFORMATION OF PNEUMOCOCCUS IN VIVO

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The phenomenon of the transformation of pneumococcal capsular types was observed first in *in vivo* experiments performed by Griffith (1). Inoculating mice subcutaneously with a mixture of living, unencapsulated pneumococci derived from a strain of one capsular type and of heat-killed encapsulated organisms of a different capsular type, he was able to recover from mice succumbing to infection living encapsulated pneumococci of the same capsular type as the heat-killed encapsulated organisms apparently inducing the change. The validity of this experiment rested upon the demonstrable absence of viable cells from the vaccine of encapsulated pneumococci. With the recognition of the type-specific M proteins of pneumococcus (2) and of the fact that they vary independently of type-specific capsular polysaccharides (3), a method for the study of pneumococcal transformation reactions *in vivo* based upon antigenic recombination has become available. The demonstration of new combinations of M protein and capsular carbohydrate in pneumococci recovered from animals inoculated experimentally with transforming mixtures establishes with certainty the fact that transformation has occurred and excludes the possibility that viable encapsulated organisms persisting in the transforming vaccine led to the illness or death of the experimental animal.

The possible occurrence of pneumococcal transformation reactions under natural circumstances *in vivo* is of interest for several reasons. Analysis of the antigenic structure of pneumococci recovered from humans ill with lobar pneumonia reveals that strains of the same capsular type may be associated with different M proteins and that strains with the same M protein may be of different capsular types. These findings suggest that antigenic recombination may take place in nature. The more recent observations of Ephrussi-Taylor (4) on the genetic interaction of phenotypically similar, yet genotypically distinct, capsular variants of pneumococcus type III have epidemiologic implications which also make desirable further knowledge regarding pneumococcal transformation reactions *in vivo*. In these experiments, the reaction between the genetically active material of one strain of pneumococcus producing very small amounts of type III capsular polysaccharide with the cells of another phenotypically similar but genetically different strain led to the emergence of a fully

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encapsulated strain of pneumococcus type III. This finding suggests that interaction between strains of low virulence may result in the appearance of a strain of heightened virulence, for it has been shown that virulence in pneumococcus is related directly to the quantity of capsular polysaccharide produced by the cell (5, 6, 7). Thus a mechanism has been demonstrated which may result in alteration of host-parasite relationships if transformation reactions do occur in nature. That very large numbers of bacteria are not necessary for the completion of the transformation reaction has been demonstrated recently by Hotchkiss (8) who has effected transformation with penicillin lysates of 10^5 pneumococcal cells.

The observations noted indicate the desirability of determining whether or not pneumococcal transformation reactions will occur in other mammalian species than the mouse and of investigating the bodily sites in which the reaction will take place.

MATERIALS AND METHODS

1. *Transformation Reactions in Vivo.*—The technique employed was that described originally by Griffith (1) as modified by MacLeod and Krauss (5). The quantity of the transforming mixture injected varied with the species of animal and the site of inoculation. Routine tests for the sterility of the transforming vaccines revealed all to be free of viable organisms.

2. *Species of Animal.*—

Mouse: white mice of the CFW strain.
Rat: white rats of the CF strain.
Guinea pig: stock laboratory animals of mixed pedigree.
Rabbit: stock laboratory animals of mixed pedigree.
Cat: domestic cats of mixed pedigree.
Monkey: *Macacus rhesus.*

3. *Strains of Pneumococcus.*—R36NC: an unencapsulated strain of pneumococcus derived originally from the fully encapsulated type II strain D39S. This strain produces the somatic M protein type 2′ and has strong alpha-hemolytic properties when grown on neopeptone-rabbit blood agar and on trypticase soy-human blood agar.

I-SVI: a fully encapsulated strain of pneumococcus type I[1] carried for many years in the laboratory. It produces the somatic M protein type 1 and gives rise to weakly alpha-hemolytic reactions on neopeptone-rabbit blood agar and on trypticase soy-human blood agar.

III-A66: a fully encapsulated strain of pneumococcus type III carried for many years in the laboratory. It produces somatic M protein type 3 and has weak hemolytic properties.

XIX: a fully encapsulated strain of pneumococcus type XIX, isolated from a patient with lobar pneumonia at the Johns Hopkins Hospital in 1951. The M protein of this strain has not been studied.

4. *Strains of Virus.*—Pneumonia Virus of Mice: PVM strain 15 isolated at the Rocke-

[1] The capsular typing sera used in this study were generously supplied by Lederle Laboratories Division, American Cyanamid Company through the courtesy of Dr. H. D. Piersma.

feller Institute for Medical Research. Mice were inoculated intranasally during light ether anaesthesia with 0.05 cc. of appropriate dilutions of suspensions of infected mouse lung.

Influenza A: strain PR8. Mice were inoculated intranasally during light ether anaesthesia with 0.03 cc. of appropriate dilutions of infected allantoic fluid.

The pneumococcal transforming mixture was administered to mice five days after infection with either virus.

5. *Preparation of Anti-M Sera, M extracts, and Techniques of Precipitin and Agglutination Tests.*—The methods used were those employed by Austrian and MacLeod (2).

EXPERIMENTAL

Pneumococcal Transformation in the Mouse.—White mice of the CFW strain were inoculated subcutaneously with 1.0 cc. of a mixture of 1 part living culture of pneumococcus R36NC and of 4 parts heat-killed transforming vaccine of pneumococcus I-SVI or III-A66. Of ten mice inoculated with the vaccine of pneumococcus I-SVI, eight succumbed to type I pneumococcal sepsis and of ten mice injected with a mixture containing the transforming vaccine derived from pneumococcus III-A66, two died of infection with pneumococcus type III. Of the type I strains recovered, seven possessed the M protein type 2′ of strain R36NC as did one of the two type III strains, demonstrating thereby antigenic recombination. The remaining type I and type III strains were found, on antigenic analysis, to possess the M protein types 1 and 3 respectively. When the hemolytic properties of the two latter strains were contrasted on the same blood agar plates with the hemolytic properties of pneumococcus R36NC and of the strains from which the transforming vaccines had been made, it was observed that the strains recovered from the mice possessed the strongly hemolytic properties of pneumococcus R36NC and differed sharply in this respect from the weakly hemolytic strains, I-SVI and III-A66. These findings, in the absence of demonstrable viable organisms in the transforming vaccines, leave little doubt that in these strains, double transformations had taken place with the acquisition both of type-specific capsular polysaccharide and of type-specific somatic M protein. The results are recorded in Table I.

A single attempt to induce capsular type transformation by injecting subcutaneously a mixture of live pneumococcus R36NC and of a partially purified solution of pneumococcal transforming principle prepared from strain III-A66 according to the method of MacLeod and Krauss (5) was unsuccessful. No encapsulated pneumococci were recovered from mice which received one hundred thousand times the amount of soluble transforming principle required to induce transformation *in vitro*.

Because the natural habitat of the pneumococcus is the respiratory tract, attempts were made to effect transformation therein. Normal mice and mice infected with the pneumonia virus of mice (PVM strain 15) or with the PR8 strain of virus influenza A were inoculated intranasally five days after initiation of the viral infection with 0.03 cc. of a transforming mixture containing

live cells of pneumococcus R36NC and a heat-killed vaccine of pneumococcus types I or III. Inasmuch as this small quantity of the transforming mixture failed to result in capsular type transformation when injected subcutaneously, it is not surprising that negative results were observed following intranasal instillation of this amount into mice with normal or diseased lungs. Control subcutaneous inoculations with larger quantities of the same mixtures did result in capsular type transformation in mice but the use of such volumes intranasally was precluded by their asphyxial effect. Nasal cultures of animals

TABLE I

Transformation In Vivo *of Pneumococcus R36NC**

MAMMALIAN SPECIES	ANTIGENS OF INDUCING STRAIN			PROPERTIES OF TRANSFORMED STRAIN		
	Strain	M protein	SSS	Hemolysis	M protein	SSS
Mouse	I-SVI	1	I	R36NC R36NC	2′ 1	I I
	III-A66	3	III	R36NC R36NC	2′ 3	III III
Rat	I-SVI	1	I	R36NC R36NC	2′ 1	I I
Guinea pig	XIX	—	XIX	R36NC	2′	XIX
Rabbit	I-SVI	1	I	R36NC R36NC	2′ 1	I I
Cat	I-SVI	1	I	R36NC	2′	I
Monkey	I-SVI	1	I	R36NC R36NC	2′ 1	I I

* This strain produces M protein type 2′ and has strong α-hemolytic properties. Strains I-SVI and III-A66 are very weakly hemolytic.

surviving intranasal inoculation of 0.03 cc. of the transforming mixture yielded small numbers of pneumococcus R36NC one, two and four weeks after inoculation. These organisms were shown to possess the type-specific protein M2′ of strain R36NC and to be competent in *in vitro* transformation reactions. The results indicate that unencapsulated pneumococci capable of acquiring a capsule through transformation may persist in the murine respiratory tract as long as four weeks.

Pneumococcal Transformation in the Rat.—Five albino rats of the CF strain were inoculated subcutaneously with 2.0 cc. of a transforming mixture of live pneumococcus R36NC and a heat-killed vaccine of pneumococcus I-SVI. Four days later, one animal succumbed to a generalized infection with bilateral

pneumonia, empyema and peritonitis due to pneumococcus type I. Determination of the somatic M protein of this strain revealed it to be type 2′, demonstrating antigenic recombination. Another strain of pneumococcus type I was recovered from the site of inoculation of the transforming mixture into a second rat sacrificed four days after injection. This strain was found to possess type 1 M protein together with the strongly hemolytic properties of pneumococcus R36NC which are not manifested by pneumococcus I-SVI. Inasmuch as no viable organisms were demonstrated in the transforming vaccine, it appears that transformation of pneumococcal M protein may take place in the rat as well as in the mouse. The remaining three rats were sacrificed two weeks after inoculation at which time they appeared well. No pneumococci were recovered from these animals, cultures from the site of inoculation proving sterile.

Pneumococcal Transformation in the Guinea Pig.—Four guinea pigs were inoculated subcutaneously with 3.0 cc. of a mixture of live pneumococcus R36NC and heat-killed transforming vaccine of pneumococcus type XIX. Pneumococcus type XIX was used because of the known susceptibility of guinea pigs to infection with this organism (9). The M protein of the strain employed was not identified but it gave no reaction with M2′ antisera. All four animals developed fever following inoculation. Culture of the site of inoculation of one animal sacrificed on the eighth day of the experiment yielded only pneumococcus R36NC. A second animal, sacrificed on the eighteenth day, had at that time a large abscess in its abdominal wall and areas of pulmonary consolidation from both of which sites type XIX pneumococci were recovered. On the twenty-second day of the experiment, a third guinea pig appeared moribund and was sacrificed. Type XIX pneumococci were grown from a small abscess in the abdominal wall. Antigenic analysis of the strains of type XIX pneumococcus recovered from the two guinea pigs showed both to possess type 2′ M protein in combination with type XIX capsular polysaccharide. No bacteria were isolated from the fourth guinea pig which died on the nineteenth day of the experiment.

Pneumococcal Transformation in the Rabbit.—Subcutaneous inoculation of four rabbits with 4.0 cc. of a mixture of live pneumococcus R36NC and a transforming vaccine of pneumococcus I-SVI resulted in the death of all four animals from type I pneumococcal septicemia within five days. The M protein extracts of heart blood cultures obtained from these animals reacted both with anti-M2′ and anti-M1 sera, a finding suggesting that the cultures were not homogeneous. When extracts were made from single clones isolated from the original heart blood cultures, they reacted either with anti-M2′ or anti-M1 serum but not with both. From three of the four rabbits, clones of type I pneumococci demonstrating antigenic recombination by virtue of their possession of type 2′ M protein were obtained. From the fourth rabbit, the only

clones examined possessed type 1 M protein. All the clones examined had the hemolytic properties of pneumococcus R36NC, differing markedly in this respect from pneumococcus I-SVI. It is of interest that from one rabbit, separate clones of type I pneumococcus possessing either type 1 or type 2′ M protein were isolated. This observation demonstrates that at least two cells underwent transformation in the same animal and that both capsular polysaccharide and somatic M protein may be acquired through transformation reactions in the rabbit.

Attempts were made to induce pneumococcal capsular type transformation in the respiratory tract of the rabbit by inoculating animals intratracheally with 4.0 cc. of the same transforming mixture used for subcutaneous inoculation. In none of five animals so injected was transformation demonstrated.

Pneumococcal Transformation in the Cat.—Subcutaneous inoculation of each of four adult domestic cats with 5.0 cc. of a mixture of live pneumococcus R36NC and transforming vaccine of pneumococcus I-SVI failed to result in the demonstration of pneumococcal transformation. Unencapsulated pneumococci were recovered from one animal from the site of injection three days after inoculation but not from the other three.

Inoculation subcutaneously of two kittens weighing approximately 500 grams each with 5.0 cc. of the same transforming mixture used to inject the adult cats resulted in the death of one animal and in an acute illness with convulsions in the other which was sacrificed twenty-four hours after infection. Intermediate capsular variants of pneumococcus type I were recovered from the local lesions and heart blood of both animals and from the spinal fluid of the animal with convulsions. These strains gave a small but definite quellung reaction with type I capsular antiserum by which they were agglutinated also. Both strains grew diffusely in anti-R serum. M protein extracts of both strains showed each to possess type 2′ M protein, demonstrating thereby antigenic recombination.

Pneumococcal Transformation in the Rhesus Monkey.—One male and one female rhesus monkey (*M. rhesus*) weighing approximately three kilograms each were inoculated subcutaneously with 3.0 cc. and 5.0 cc. respectively of a transforming mixture of live pneumococcus R36NC and heat-killed vaccine of pneumococcus I-SVI. Three weeks previously, the male animal had been inoculated intrabronchially[2] with 3.0 cc. of the same mixture following which procedure type I pneumococci had not been recovered by transthoracic puncture of the injected lung. Two days after subcutaneous inoculation, both animals were febrile and heart blood cultures revealed both monkeys to have type I pneumococcal septicemia from which they recovered spontaneously. In addition, aspiration of abscesses at the site of subcutaneous inoculation

[2] Appreciation is expressed to Dr. Donald F. Proctor for performing this procedure.

yielded type I pneumococci. Clones of type I pneumococci from the heart blood culture of each animal were examined and each was found to possess type 2′ M protein. Clones from the subcutaneous abscess of each animal, however, were found to be composed of cells containing type 1 M protein, yet manifesting the strongly hemolytic properties of pneumococcus R36NC. The results demonstrate that transformation of pneumococcal capsular type and M protein may take place in a primate and that more than one bacterial cell may be transformed in the same animal.

DISCUSSION

The experimental data reveal that the phenomenon of pneumococcal transformation is one that may be demonstrated in a variety of mammalian species including several rodents, a carnivore and a primate. The conditions used to induce pneumococcal transformation *in vivo*, however, are highly artificial and unlikely to be reproduced under natural circumstances. Inability to demonstrate transformation in the respiratory tract, the natural habitat of pneumococcus, may not indicate anything other than the fact that the experimental procedures employed were inappropriate; and a number of factors may have contributed to the failure of the experiments. Study of pneumococcal transformation *in vitro* reveals that a serum factor (10), present in bovine serum albumin (11), is essential to the completion of the reaction. It has been shown also, however, that mammalian sera manifest desoxyribonuclease activity which inhibits transformation by inactivation of the transforming principle (12). At present nothing is known regarding how the balance of these two antagonistic factors may influence transformation *in vivo*. Use of a heat-killed pneumococcal vaccine as a source of transforming principle is probably inefficient, for the destruction of the cells is slowed markedly by the inactivation of their autolytic enzymes and the amount of desoxyribonucleic acid released is doubtless small. The number of cells in the volume of transforming vaccine required for these experiments was more than one hundred thousand times the number necessary to yield an active lysate in the *in vitro* experiments of Hotchkiss (8). The bacterial vaccine, however, appears to provide local environmental conditions which are not duplicated by the use *in vivo* of soluble transforming principle in large excess, for transformation was not observed when the latter was employed. Another factor which may operate to influence the outcome of transformation reactions in the respiratory tract is the susceptibility of the transforming principle to reversible oxidative inactivation (13). This fact may make the lungs potentially a less suitable site than the paranasal sinuses for transformation reactions. Finally, the role of leukocytes in phagocytizing living unencapsulated cells in the transforming mixture requires consideration. In so vascular a structure as the lung, the accessibility of the unencapsulated

pneumococci to phagocytes which can engulf these invading bacteria readily in the absence of added antibody may interfere with transformation.

It appears, therefore, that though a considerable body of evidence supports indirectly the idea that pneumococcal transformation reactions may take place in nature and though the present studies reveal that the reaction may occur under artificial circumstances in a variety of mammalian species, the natural conditions under which this reaction may occur still await definition.

SUMMARY

The transformation of pneumococcal capsular polysaccharide and type-specific somatic M protein in a variety of mammalian species is described and the relation of these observations to the possible natural occurrence of pneumococcal transformation reactions is discussed.

BIBLIOGRAPHY

1. GRIFFITH, F.: The significance of pneumococcal types. J. Hyg., 1928, **27**: 113.
2. AUSTRIAN, R. AND MACLEOD, C. M.: A type-specific protein from pneumococcus. J. Exp. Med., 1949, **89**: 439.
3. AUSTRIAN, R. AND MACLEOD, C. M.: Acquisition of M protein by pneumococcus through transformation reactions. J. Exp. Med., 1949, **89**: 451.
4. EPHRUSSI-TAYLOR, H.: Transformations allogènes du pneumocoque. Exp. Cell Research, 1951, **2**: 589.
5. MACLEOD, C. M. AND KRAUSS, M. R.: Stepwise intratype transformation of pneumococcus from R to S by way of a variant intermediate in capsular polysaccharide production. J. Exp. Med., 1947, **86**: 439.
6. MACLEOD, C. M. AND KRAUSS, M. R.: Relation of the virulence of pneumococcal strains for mice to the quantity of capsular polysaccharide formed in vitro. J. Exp. Med., 1950, **92**: 1.
7. WOOD, W. B., JR. AND SMITH, M. R.: Host-parasite relationships in experimental pneumonia due to Pneumococcus type III. J. Exp. Med., 1950, **92**: 85.
8. HOTCHKISS, R. D.: Transfer of penicillin-resistance in pneumococci by desoxyribonucleate fractions from resistant cultures. Cold Spring Harbor Symposia on Quant. Biol., 1951, **16**: 457.
9. FINLAND, M.: Recent advances in the epidemiology of pneumococcal infections. Medicine, 1942, **21**: 307.
10. MCCARTY, M., TAYLOR, H. E. AND AVERY, O. T.: Biochemical studies of environmental factors essential in transformation of pneumococcal types. Cold Spring Harbor Symposia on Quant. Biol., 1946, **11**: 177.
11. HOTCHKISS, R. D. AND EPHRUSSI-TAYLOR, H.: Use of serum albumin as a source of serum factor in pneumococcal transformation. Federation Proc., 1951, **10**: 200.
12. AVERY, O. T., MACLEOD, C. M. AND MCCARTY, M.: Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleate fraction isolated from Pneumococcus type III. J. Exp. Med., 1944, **79**: 137.
13. MCCARTY, M.: Reversible inactivation of the substance inducing transformation of pneumococcal types. J. Exp. Med., 1945, **81**: 501.